CLAIMS

1. A fluorescent protein having chemiluminescence activity.

2. The fluorescent protein having chemiluminescence activity of claim 1, wherein the fluorescence spectrum of fluorescence radiated during emission of the fluorescence is identical to the emission spectrum of light radiated during chemiluminescence.

3. A method for emitting light, wherein a luminescent substrate is added to a fluorescent protein having chemiluminescence activity.

4. A method for detecting a target substance,

wherein a fluorescent protein having chemiluminescence activity, to which a ligand for the target substance has been bound, is bound to the target substance via the ligand,

and caused to emit light by adding a luminescent substrate,

so that radiated light is detected.

5. A fluorescent protein having chemiluminescence activity, to which a ligand for a target substance to be detected is bound.

6. A fluorescent protein having chemiluminescence activity, comprising an apoprotein that is a component of a calcium-binding photoprotein, a coelenteramid or an analog thereof, and a calcium ion or a divalent or trivalent ion that can be substituted for the calcium ion.

7. The fluorescent protein having chemiluminescence activity of claim 6, wherein, in the complex of the apoprotein and the coelenteramid or the analog thereof, the ratio of the number of molecules of the apoprotein to the number of molecules of the coelenteramid or the analog thereof is 1:1; and wherein, in the complex of the apoprotein and the calcium ion or the divalent or trivalent ion that can be substituted for the calcium ion, the ratio of the number of molecules of the apoprotein to the number of molecules of the calcium ion or the divalent or trivalent ion is 1:1 to 1:4.

8. The fluorescent protein having chemiluminescence

activity of claim 6, wherein the apoprotein is an protein selected from the group consisting of apoaequorin, apoclytin, apoobelin, apomitrocomin, apomineopsin, and apobervoin.

9. The fluorescent protein having chemiluminescence activity of claim 6, wherein the apoprotein is an apoaequorin having the amino acid sequence shown in SEQ ID NO: 1 or a mutant apoaequorin in which one or more amino acids are deleted, substituted, or added in the sequence shown in SEQ ID NO: 1.

10. The fluorescent protein having chemiluminescence activity of claim 6, wherein the apoprotein is an apoobelin having the amino acid sequence shown in SEQ ID NO: 2 or a mutant apoobelin in which one or more amino acids are deleted, substituted, or added in the sequence shown in SEQ ID NO: 2.

11. The fluorescent protein having chemiluminescence activity of claim 6, wherein the apoprotein is an apoclytin having the amino acid sequence shown in SEQ ID NO: 3 or a mutant apoclytin in which one or more amino acids are deleted, substituted, or added in the sequence shown in SEQ ID NO: 3.

12. The fluorescent protein having chemiluminescence activity of claim 6, wherein the apoprotein is an apomitrocomin having the amino acid sequence shown in SEQ ID NO: 4 or a mutant apomitrocomin in which one or more amino acids are deleted, substituted, or added in the sequence shown in SEQ ID NO: 4.

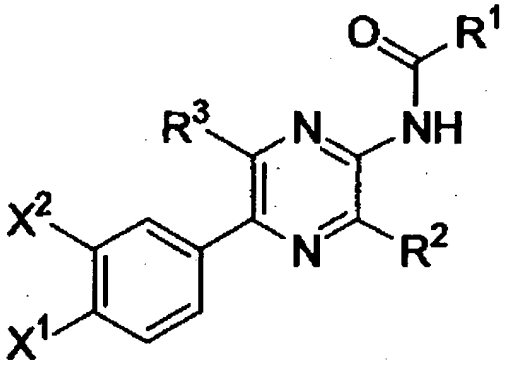
13. A fluorescent protein muntant having chemiluminescence activity, comprising an apoprotein that is a component of a calcium-binding photoprotein, a coelenteramid or an analog thereof, and a calcium ion or a divalent or trivalent ion that can be substituted for the calcium ion,

the mutant comprising a mutated apoprotein in which at least one of at least two free sulfhydryl groups which make(s) its chemiluminescence activity lost on formation of their disulfide bond, possessed by the apoprotein of the fluorescent protein is deleted or substituted, so that formation disulfide bonds cannot be formed.

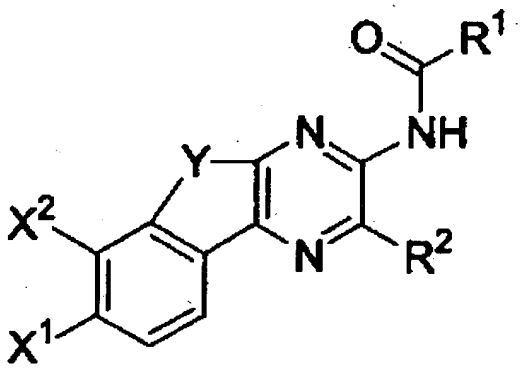
14. A fluorescent protein having chemiluminescence activity, comprising an apoprotein that is a component of a

calcium-binding photoprotein, a coelenteramid or an analog thereof, and a calcium ion or a divalent or trivalent ion that can be substituted for the calcium ion,

wherein the coelenteramid or the analog thereof is represented by the following formula (1) or (2):



(1)



(2)

wherein

R^1 is a substituted or unsubstituted aryl group, a substituted or unsubstituted arylated alkyl group, or a straight or branched chain alkyl group that may be substituted by an aliphatic cyclic group;

R^2 is a substituted or unsubstituted aryl group, a substituted or unsubstituted arylated alkyl group, a substituted or unsubstituted aryl alkenyl group, a straight or branched chain alkyl group that may be substituted by an aliphatic cyclic group, a straight or branched chain alkenyl group that may be substituted by an aliphatic cyclic group, or

a heterocyclic group;

R^3 is a hydrogen atom, a substituted or unsubstituted alkyl group;

X^1 is a hydrogen atom, a hydroxyl group, a halogen atom, an alkoxyl group, or an amino group;

X^2 is a hydrogen atom or a hydroxyl group; and

Y is a divalent hydrocarbon group having 1 to 4 carbon atoms.

15. The fluorescent protein having chemiluminescence activity of claim 14,

wherein, in the formula (1) or (2),

R^1 is an unsubstituted aryl group, an unsubstituted arylated alkyl group, an arylated alkyl group substituted by a hydroxyl group or a halogen atom, or a straight or branched chain alkyl group that may be substituted by a cyclohexyl group;

R^2 is an unsubstituted aryl group, an aryl group substituted by a hydroxyl group, an unsubstituted arylated alkyl group, an arylated alkyl group substituted with a hydroxyl group, an unsubstituted aryl alkenyl group, an unsubstituted straight or branched chain alkyl group, a straight chain alkyl group that may be substituted by an aliphatic cyclic group, a branched chain alkenyl group, a heterocyclic group containing sulfur;

R^3 is a hydrogen atom, a methyl group, or 2-hydroxyethyl group;

X^1 is a hydrogen atom, a hydroxyl group, a fluorine atom, a methoxy group, or an amino group; and

Y is a methylene group, ethylene group, a propylene group, or a vinylene group.

16. The fluorescent protein (bFP) having chemiluminescence activity of claim 14,

wherein, in the formula (1) or (2),

R^1 is a phenyl group, a benzyl group, a p-hydroxybenzyl group, a p-fluorobenzyl group, a p-chlorobenzyl group, a p-bromobenzyl group, a p-iodinebenzyl group, a 3, 4-difluorobenzyl group, a pentafluorobenzyl group, a

phenylethyl group, a phenylpropyl group, a naphthylmethyl group, a cyclohexylmethyl group, a methyl group, a 1-methylpropyl group, or a 2-methylpropyl group; and

R^2 is a phenyl group, a p-hydroxy phenyl group, a benzyl group, an <alpha>-hydroxybenzyl group, a phenylethyl group, a phenylvinyl group, a cyclohexyl group, a cyclohexylmethyl group, a cyclohexylethyl group, a methyl group, an ethyl group, a propyl group, a 2-methylpropyl group, a 2-methylpropenyl group, an adamantylmethyl group, a cyclopentylmethyl group, or a thiophene-2-yl group.

17. The fluorescent protein having chemiluminescence activity of claim 6, wherein the calcium ion or the divalent trivalent ion that can be substituted for the calcium ion is one selected from the group consisting of a calcium ion, a strontium ion, and a lead ion.

18. A method for detecting a target substance, comprising:

a step of binding a ligand for the target substance to a fluorescent protein having chemiluminescence activity comprising an apoprotein that is a component of a calcium-binding photoprotein, a coelenteramid or an analog thereof, and a calcium ion or a divalent or trivalent ion that can be substituted for the calcium ion,

a step of binding the fluorescent protein to the target substance to be detected via the ligand

a step of emiting light by adding a coelenterazine or an analog thereof, and

a step of detecting the emitted light.

19. A fluorescent protein having chemiluminescence activity to which a ligand for a target substance to be detected is bound,

comprising an apoprotein that is a component of a calcium-binding photoprotein, a coelenteramid or an analog hereof, and a calcium ion or a divalent or trivalent ion that can be substituted for the calcium ion.

20. A method for enhancing thermal stability of a fluorescent protein having chemiluminescence activity comprising an apoprotein that is a component of a calcium-binding

photoprotein, a coelenteramid or an analog thereof, and a calcium ion or a divalent or trivalent ion that can be substituted for the calcium ion,

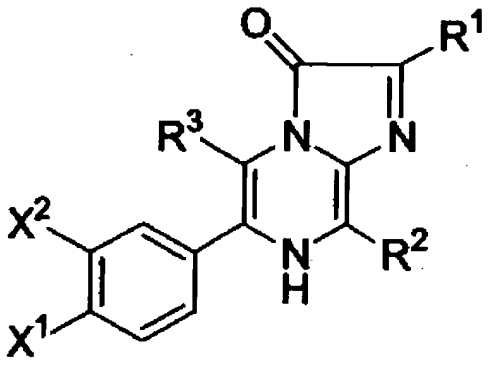
wherein a reducing agent is added to a solution in which the fluorescent protein is dissolved.

21. The method for enhancing thermal stability of claim 20, wherein the reducing agent is dithiothreitol or mercaptoethanol.

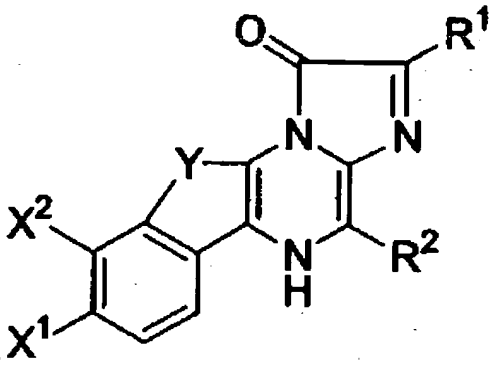
22. A chemiluminescence method, comprising a step of making a coelenterazine or an analog thereof to react with a fluorescent protein comprising an apoprotein that is a component of a calcium-binding photoprotein, a coelenteramid or an analog thereof, and a calcium ion or a divalent or trivalent ion that can be substituted for the calcium ion.

23. The chemiluminescence method of claim 22, wherein the coelenterazine or the analog thereof is made to be react in the presence of a reducing agent.

24. The chemiluminescence method of claim 22, wherein the coelenterazine or the analog thereof is represented in the following formula (3) or (4):



(3)



(4)

wherein

R^1 is a substituted or unsubstituted aryl group, a substituted or unsubstituted arylated alkyl group, or a straight or branched chain alkyl group that may be substituted by an aliphatic cyclic group;

R^2 is a substituted or unsubstituted aryl group, a substituted or unsubstituted arylated alkyl group, a substituted or unsubstituted aryl alkenyl group, a straight or branched chain alkyl group that may be substituted by an aliphatic cyclic group, a straight or branched chain alkenyl group that may be substituted by an aliphatic cyclic group, or a heterocyclic group;

R^3 is a hydrogen atom, a substituted or unsubstituted alkyl group;

X^1 is a hydrogen atom, a hydroxyl group, a halogen atom, an alkoxyl group, or an amino group;

X^2 is a hydrogen atom or a hydroxyl group; and

Y is a divalent hydrocarbon group having 1 to 4 carbon atoms.

25. The chemiluminescence method of claim 24, wherein, in the formula (3) or formula (4),

R^1 is an unsubstituted aryl group, an unsubstituted arylated alkyl group, an arylated alkyl group substituted by a hydroxyl group or a halogen atom, or a straight or branched chain alkyl group that may be substituted by a cyclohexyl group;

R^2 is an unsubstituted aryl group, an aryl group

substituted by a hydroxyl group, an unsubstituted arylated alkyl group, an arylated alkyl group substituted with a hydroxyl group, an unsubstituted aryl alkenyl group, an unsubstituted straight or branched chain alkyl group, a straight chain alkyl group that may be substituted by an aliphatic cyclic group, a branched chain alkenyl group, a heterocyclic group containing sulfur;

R^3 is a hydrogen atom, a methyl group, or 2-hydroxyethyl group;

X^1 is a hydrogen atom, a hydroxyl group, a fluorine atom, a methoxy group, or an amino group; and

Y is a methylene group, ethylene group, a propylene group, or a vinylene group.

26. The chemiluminescence method of claim 24,

wherein, in the formula (3) or (4),

R^1 is a phenyl group, a benzyl group , a p-hydroxybenzyl group, a p-fluorobenzyl group, a p-chlorobenzyl group, a p-bromobenzyl group, a p-iodinebenzyl group, a 3, 4-difluorobenzyl group, a pentafluorobenzyl group, a phenylethyl group, a phenylpropyl group, a naphthylmethyl group, a cyclohexylmethyl group, a methyl group, a 1-methylpropyl group, or a 2-methylpropyl group; and

R^2 is a phenyl group, a p-hydroxy phenyl group, a benzyl group, an α-hydroxybenzyl group, a phenylethyl group, a phenylvinyl group, a cyclohexyl group, a cyclohexylmethyl group, a cyclohexylethyl group, a methyl group, an ethyl group, a propyl group, a 2-methylpropyl group, a 2-methylpropenyl group, an adamantylmethyl group, a cyclopentylmethyl group, or a thiophene-2-yl group.

27. A luminescence kit comprising the fluorescent protein having chemiluminescence activity of claim 6 and a coelenterazine or an analog thereof.

28. The luminescence kit of claim 27, further comprising a reducing agent.

29. A method for producing a fluorescent protein having chemiluminescence activity, comprising a step of having a

calcium-binding photoprotein comprising an apoprotein and a coelenterazine or an analog thereof react with a solution of a calcium ion or a divalent or trivalent ion that can be substituted for the calcium ion,

wherein the reaction is performed such that a coelenteramid or an analog thereof formed from the coelenterazine or the analog thereof remains coordinated into the apoprotein.

30. A method for producing a fluorescent protein having chemiluminescence activity, comprising the step of reacting a calcium-binding photoprotein comprising an apoprotein and a coelenterazine or an analog thereof with a solution of a calcium ion or a divalent or trivalent ion that can be substituted for the calcium ion,

wherein the reaction is performed such that the apoprotein is prevented from forming de novo disulfide bonds.

31. The production method of claim 29, wherein the calcium-binding photoprotein is made to react with the solution, at a concentration of 10^{-7} M or lower, of the calcium ion or the divalent or trivalent ion that can be substituted for the calcium ion.

32. A method for producing a fluorescent protein having chemiluminescence activity,

wherein a calcium ion or a divalent or trivalent ion that can be substituted for the calcium ion is made to react with a fluorescent protein comprising an apoprotein that is a component of a calcium-binding photoprotein, and a coelenteramid or an analog thereof.

33. A fluorescent protein comprising an apoprotein that is a component of a calcium-binding photoprotein, and a coelenteramid or an analog thereof.

34. The fluorescent protein of claim 33, comprising the apoprotein and the coelenteramid or the analog thereof in the same number of molecules in a complex.

35. The fluorescent protein of claim 33, wherein the apoprotein is selected from the group consisting of apoaequorin,

apoclytin, apoobelin, apomitrocomin, apomineopsin, and apobervoin.

36. The fluorescent protein having chemiluminescence activity of claim 33, wherein the apoprotein is an apoaequorin having the amino acid sequence shown in SEQ ID NO: 1 or a mutant apoaequorin in which one or more amino acids are deleted, substituted, or added in the sequence shown in SEQ ID NO: 1.

37. The fluorescent protein having chemiluminescence activity of claim 33, wherein the apoprotein is an apoobelin having the amino acid sequence shown in SEQ ID NO: 2 or a mutant apoobelin in which one or more amino acids are deleted, substituted, or added in the sequence shown in SEQ ID NO: 2.

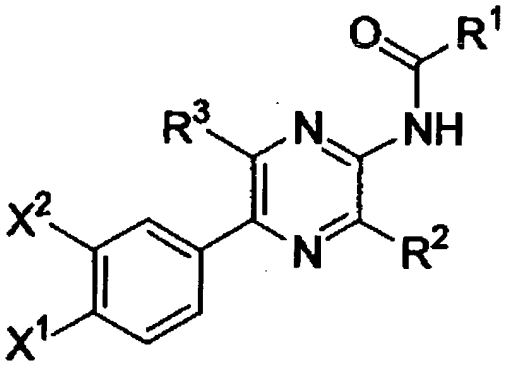
38. The fluorescent protein having chemiluminescence activity of claim 33, wherein the apoprotein is an apoclytin having the amino acid sequence shown in SEQ ID NO: 3 or a mutant apoclytin in which one or more amino acids are deleted, substituted, or added in the sequence shown in SEQ ID NO: 3.

39. The fluorescent protein having chemiluminescence activity of claim 33, wherein the apoprotein is an apomitrocomin having the amino acid sequence shown in SEQ ID NO: 4 or a mutant apomitrocomin in which one or more amino acids are deleted, substituted, or added in the sequence shown in SEQ ID NO: 4.

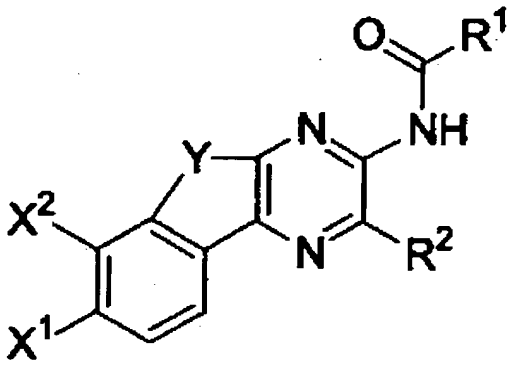
40. A fluorescent protein mutant comprising an apoprotein that is a component of a calcium-binding photoprotein, and a coelenteramid or a an analog thereof,

comprising a mutant apoprotein in which at least one of at least two free sulfhydryl groups of disulfide bonds, which make(s) their chemiluminescence activity lost on formation of disulfide bond, possessed by the apoprotein of the fluorescent protein, is deleted or substituted, so that disulfide bonds cannon be formed.

41. The fluorescent protein of claim 33, wherein the coelenteramid or the analog thereof is represented by the following formula (1) or (2):



(1)



(2)

wherein

R^1 is a substituted or unsubstituted aryl group, a substituted or unsubstituted arylated alkyl group, or a straight or branched chain alkyl group that may be substituted by an aliphatic cyclic group;

R^2 is a substituted or unsubstituted aryl group, a substituted or unsubstituted arylated alkyl group, a substituted or unsubstituted aryl alkenyl group, a straight or branched chain alkyl group that may be substituted by an aliphatic cyclic group, a straight or branched chain alkenyl group that may be substituted by an aliphatic cyclic group, <or> a heterocyclic group;

R^3 is a hydrogen atom, a substituted or unsubstituted alkyl group;

X^1 is a hydrogen atom, a hydroxyl group, a halogen atom,

an alkoxyl group, or an amino group;

X^2 is a hydrogen atom or a hydroxyl group; and

Y is a divalent hydrocarbon group having 1 to 4 carbon atoms.

42. The fluorescent protein of claim 41,

wherein, in the formula (1) or (2),

R^1 is an unsubstituted aryl group, an unsubstituted arylated alkyl group, an arylated alkyl group substituted by a hydroxyl group or a halogen atom, or a straight or branched chain alkyl group that may be substituted by a cyclohexyl group;

R^2 is an unsubstituted aryl group, an aryl group substituted by a hydroxyl group, an unsubstituted arylated alkyl group, an arylated alkyl group substituted with a hydroxyl group, an unsubstituted aryl alkenyl group, an unsubstituted straight or branched chain alkyl group, a straight chain alkyl group that may be substituted by an aliphatic cyclic group, a branched chain alkenyl group, a heterocyclic group containing sulfur;

R^3 is a hydrogen atom, a methyl group, or 2-hydroxyethyl group;

X^1 is a hydrogen atom, a hydroxyl group, a fluorine atom, a methoxy group, or an amino group; and

Y is a methylene group, ethylene group, a propylene group, or a vinylene group.

43. The fluorescent protein of claim 41,

wherein, in the formula (1) or (2),

R^1 is a phenyl group, a benzyl group , a p-hydroxybenzyl group, a p-fluorobenzyl group, a p-chlorobenzyl group, a p-bromobenzyl group, a p-iodinebenzyl group, a 3, 4-difluorobenzyl group, a pentafluorobenzyl group, a phenylethyl group, a phenylpropyl group, a naphthylmethyl group, a cyclohexylmethyl group, a methyl group, a 1-methylpropyl group, or a 2-methylpropyl group; and

R^2 is a phenyl group, a p-hydroxy phenyl group, a benzyl group, an α-hydroxybenzyl group, a phenylethyl group, a phenylvinyl group, a cyclohexyl group, a cyclohexylmethyl group,

a cyclohexylethyl group, a methyl group, an ethyl group, a propyl group, a 2-methylpropyl group, a 2-methylpropenyl group, an adamantylmethyl group, a cyclopentylmethyl group, or a thiophene-2-yl group.

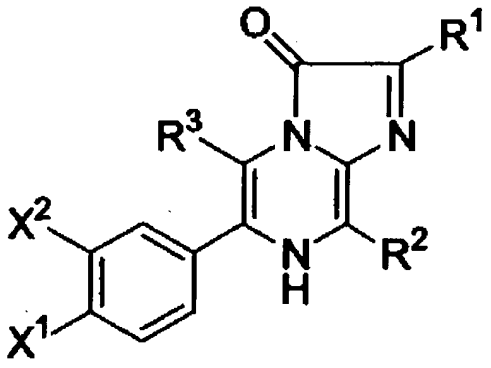
44. The fluorescent protein of claim 33, to which a ligand for a target substance to be detected is bound.

45. A method for detecting a calcium ion or an ion that can be substituted for the calcium ion, comprising a step of using a fluorescent protein comprising an apoprotein that is a component of a calcium-binding photoprotein, and a coelenteramid or an analog thereof.

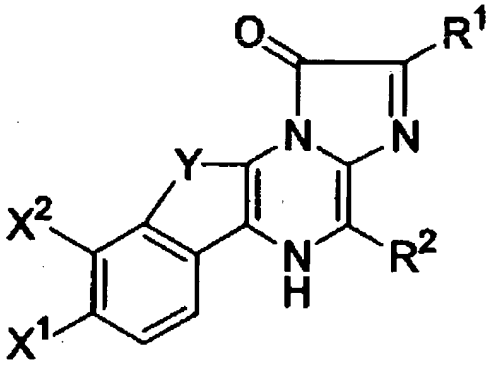
46. A method for quantifying a calcium ion or an ion that can be substituted for the calcium ion, comprising a step of using a fluorescent protein comprising an apoprotein that is a component of a calcium-binding photoprotein, and a coelenteramid or an analog thereof.

47. A method for producing a calcium-binding photoprotein comprising an apoprotein and a coelenterazine or a an analog thereof, comprising a step of making the coelenterazine or the analog thereof react with the fluorescent protein comprising the apoprotein and the coelenteramid or the analog thereof.

48. The method for producing a calcium-binding photoprotein of claim 47, wherein the coelenterazine or the analog thereof is represented in the following formula (3) or (4):



(3)



(4)

wherein

R^1 is a substituted or unsubstituted aryl group, a substituted or unsubstituted arylated alkyl group, or a straight or branched chain alkyl group that may be substituted by an aliphatic cyclic group;

R^2 is a substituted or unsubstituted aryl group, a substituted or unsubstituted arylated alkyl group, a substituted or unsubstituted aryl alkenyl group, a straight or branched chain alkyl group that may be substituted by an aliphatic cyclic group, a straight or branched chain alkenyl group that may be substituted by an aliphatic cyclic group, <or> a heterocyclic group;

R^3 is a hydrogen atom, a substituted or unsubstituted alkyl group;

X^1 is a hydrogen atom, a hydroxyl group, a halogen atom, an alkoxyl group, or an amino group;

X^2 is a hydrogen atom or a hydroxyl group; and

Y is a divalent hydrocarbon group having 1 to 4 carbon atoms.

49. The method for producing a calcium-binding photoprotein of claim 48,

wherein, in the formula (3) or (4),

R^1 is an unsubstituted aryl group, an unsubstituted arylated alkyl group, an arylated alkyl group substituted by a hydroxyl group or a halogen atom, or a straight or branched chain alkyl group that may be substituted by a cyclohexyl group;

R^2 is an unsubstituted aryl group, an aryl group substituted by a hydroxyl group, an unsubstituted arylated alkyl group, an arylated alkyl group substituted with a hydroxyl group, an unsubstituted aryl alkenyl group, an unsubstituted straight or branched chain alkyl group, a straight chain alkyl group that may be substituted by an aliphatic cyclic group, a branched chain alkenyl group, a heterocyclic group containing sulfur;

R^3 is a hydrogen atom, a methyl group, or 2-hydroxyethyl group;

X^1 is a hydrogen atom, a hydroxyl group, a fluorine atom, a methoxy group, or an amino group; and

Y is a methylene group, ethylene group, a propylene group, or a vinylene group.

50. The method for producing a calcium-binding photoprotein of claim 48,

wherein, in the formula (3) or (4),

R^1 is a phenyl group, a benzyl group , a p-hydroxybenzyl group, a p-fluorobenzyl group, a p-chlorobenzyl group, a p-bromobenzyl group, a p-iodinebenzyl group, a 3, 4-difluorobenzyl group, a pentafluorobenzyl group, a phenylethyl group, a phenylpropyl group, a naphthylmethyl group, a cyclohexylmethyl group, a methyl group, a 1-methylpropyl group, or a 2-methylpropyl group; and

R^2 is a phenyl group, a p-hydroxy phenyl group, a benzyl group, an α-hydroxybenzyl group, a phenylethyl group, a phenylvinyl group, a cyclohexyl group, a cyclohexylmethyl group, a cyclohexylethyl group, a methyl group, an ethyl group, a propyl group, a 2-methylpropyl group, a 2-methylpropenyl group, an adamantylmethyl group, a cyclopentylmethyl group, or a thiophene-2-yl group.

51. The method for producing a calcium-binding photoprotein of claim 47, wherein the coelenterazine or the analog thereof is made to react with the fluorescent protein in the presence of a reducing agent.

52. A kit for producing a fluorescent protein comprising an

apoprotein that is a component of a calcium-binding photoprotein, and a coelenteramid or an analog thereof; and (b) a calcium-binding photoprotein containing a coelenterazine or an analog thereof.

53. The kit for producing a calcium-binding photoprotein of claim 52, further comprising a reducing agent.

54. A method for detecting a target substance,comprising:

a step of binding a fluorescent protein, to which a ligand for the target substance has been bound, comprising an apoprotein that is a component of a calcium-binding photoprotein, and a coelenteramid or an analog thereof to the target substance via the ligand,

a step of adding a coelenterazine or a an analog thereof,

a step of emitting light by adding a calcium ion or a divalent or trivalent ion that can be substituted for the calcium ion.

55. A method for producing a fluorescent protein comprising an apoprotein that is a component of a calcium-binding photoprotein, and a coelenteramid or an analog thereof, comprising:

a step of removing a calcium ion or a divalent or trivalent ion that can be substituted for the calcium ion by adding a chelating agent to the fluorescent protein having chemiluminescence activity, comprising the apoprotein, the coelenteramid or the analog thereof, and the calcium ion or the divalent or trivalent ion that can be substituted for the calcium ion.

56. A method for producing a calcium-binding photoprotein comprising an apoprotein and a coelenterazine or an analog thereof, comprising a step of making the coelenterazine or the analog thereof react with a fluorescent protein comprisnig the apoprotein, a coelenteramid or an analog thereof, and a calcium ion or a divalent or trivalent ion that can be substituted for a calcium ion in the presence of a chelating agent for removing the calcium ion or the divalent or trivalent ion that can be substituted for the calcium ion.